pDRIVE-mTRP
A plasmid encoding the mouse TRP1 promoter
http://www.invivogen.com/pdrive-trp1
Catalog # pdrive-mtrp

For research use only
Version # 17J16-MM

PRODUCT INFORMATION
Contents:
• 20 µg of pDRIVE-mTRP provided as lyophilized DNA
• 4 pouches of E. coli Fast-Media® Zeo (2 TB and 2 Agar)

Storage and Stability:
• Product is shipped at room temperature.
• Lyophilized DNA should be stored at -20°C.
• Resuspended DNA should be stored at -20°C and is stable up to 1 year.
• Store E. coli Fast-Media® at room temperature in a dry and cool place.
Fast-Media® pouches are stable 2 years when stored properly.

Quality Control:
• Plasmid construct has been confirmed by restriction analysis and full-length ORF sequencing.
• Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE
pDRIVE is an expression plasmid containing a native or composite promoter of interest. pDRIVE may be used to:
- Subclone a promoter of interest into another vector. Typically, unique restriction sites are present at each end of the promoter allowing convenient excision. The 5’ restriction site is SdaI, which is unique and compatible with NsiI and PsI. As the 3’ NcoI restriction site is not unique, we recommend using another restriction such as BbsI and generating an oligo linker which includes the ATG start codon.
- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE promoter drives the expression of the LacZ reporter gene which allows for testing of the promoter’s activity in transient transfection experiments. Furthermore, the LacZ gene is flanked by unique restriction sites (NcoI and EcoRI) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS
Murine Tyrosinase Related Protein (mTRP) promoter
Complete promoter size: 1201 bp
Specificity: Melanocytes & melanoma

The TRP1 gene encodes the tyrosinase-related protein 1, an enzyme involved in the process that converts tyrosinase to melanin pigments. Expression of TRP1 is restricted to melanocytes and melanoma cells partly as a consequence of transcriptional regulation of its mRNA. The TRP1 promoter shares with the tyrosinase promoter an 11-bp motif termed the M box located upstream of the TATA box. This M box plays a key role in the regulation of the TRP1 expression by cyclic AMP (cAMP). Tissue-specific expression of transgenes, such as the IL-2 and HSV1-tk genes, driven by the TRP1 promoter has been demonstrated in murine model systems, achieving marked antitumor effects.

1. Bertolotto C. et al., 1996. Different cis-acting elements are involved in the regulation of TRP1 and TRP2 promoter activities by cyclic AMP; pivotal role of M boxes (GTCTAGTCGCT) and of microphthalmia Mol Cell Biol. 18(2):694-702.

PLASMID FEATURES
• LacZ gene encodes β-galactosidase an enzyme that catalyzes the hydrolysis of X-Gal, producing a blue precipitate that can be easily visualized under a microscope.
• SV40 pAn: The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
• pMBI Ori is a minimal E. coli origin of replication with the same activity as the longer Ori.
• EM2K is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in E. coli.
• Zeo gene confers Zeocin™ resistance therefore allowing the selection of transformed E. coli carrying a pDRIVE plasmid.

Note: Stable transfection of clones cannot be performed due to the absence of an eukaryotic promoter upstream of the Sh ble gene.

METHODS
Plasmid resuspension:
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H2O. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning:
Plasmid amplification and cloning can be preformed in E. coli GT116 other commonly used laboratory E. coli strains, such as DH5α.

Selection of bacteria with E. coli Fast-Media Zeo:
E. coli Fast-Media® Zeo is a fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave. E. coli Fast-Media® Zeo is a TB (liquid) or LB (solid) based medium with zeocin, and contains stabilizers.

E. coli Fast-Media® Zeo can be ordered separately (catalog code fas-zn-l, fas-zn-s).

Method:
1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled water to the flask
3- Heat in a microwave on MEDIUM power setting (about 400 Watts), until bubbles start appearing (approximately 3 minutes). Do not heat a closed container. Do not autoclave Fast-Media®.
4- Swirl gently to mix the preparation. Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.
5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT
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www.invivogen.com
pDRIVE01-TRP1(m) v04

SV40 p(A)

LacZ

EM7

rpmB/G term

Sh ble ΔCpG

ori pMB1

(pMB1 prom)

BbsI (4100)

PacI (4673)

NheI (4398)

EcoRI (4392)

BbsI (1153)

NcoI (1207)

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